

Appl. No. 10/798,440
Amendment dated: January 14, 2005
Reply to OA of: September 17, 2004

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims:

1(currently amended). A method for fabricating a substrate by purification, modification and immobilization of recombinant protein, said method comprising the steps of:

tagging a DNA sequence encoding a target protein into a recombinant vector with a specific tag sequence;

expressing the vector under suitable condition to obtain a recombinant protein; purifying and modifying said recombinant protein by using an affinity column and a modification reagent;

exchanging said recombinant protein which has been attached to the affinity column with a decoupling reagent; and

immobilizing said recombinant protein onto a substrate.

2(currently amended). The method as claimed in claim 1, wherein said specific tag comprises Histidine tag, Maltose-binding tag, or ~~GST~~glutathione S transferase tag.

3(original). The method as claimed in claim 2, wherein said specific genetic tag is Histidine tag.

4(original). The method as claimed in claim 1, wherein said recombinant protein is prepared by using prokaryotic cell, eukaryotic cell or an in vitro transcription/translation system.

5(original). The method as claimed in claim 4, wherein said prokaryotic cell is *E. coli*.

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6(original). The method as claimed in claim 4, wherein said eukaryotic cell is yeast, insect cell or mammalian cell.

7(original). The method as claimed in claim 1, wherein the affinity column for capturing the recombinant protein is chosen in corresponding to said specific tag.

8(original). The method as claimed in claim 7, when said specific tag is Histidine tag, a metal chelating column is used as the affinity column.

9(original). The method as claimed in claim 8, wherein the metal chelation column is represented by a general formula as metal-X column.

10(original). The method as claimed in claim 9, wherein the metal in said formula comprises nickel, zinc, copper, or cobalt.

11(original). The method as claimed in claim 9, wherein the X in said formula comprises iminodiacetic acid, nitrilotriacetic acid, tris(carboxymethyl)- ethylenediamine, carboxymethylaspartate, or TALON, a immobilized metal affinity resin.

12(currently amended). The method as claimed in claim 9, wherein the metal-X column is ~~Ni-IDA~~Ni-iminodiacetic acid column or ~~Cu-IDA~~Cu-iminodiacetic acid column.

13(original). The method as claimed in claim 7, when said specific tag is Maltose-binding tag, an amylose column is used as the affinity column.

14(original). The method as claimed in claim 7, when said specific tag is a GST-tag, glutathione column is used as the affinity column.

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15(original). The method as claimed in claim 1, wherein said recombinant protein is modified by using a biotinylation reaction so to add biotin functional groups to said recombinant protein.

16(original). The method as claimed in claim 15, wherein the modification of said recombinant protein comprising the steps of:

- obtaining a solution containing the recombinant protein;
- adding a biotinylation reagent to cause biotinylation reaction with said recombinant protein; and
- capturing said biotinlyted recombinant protein by using the affinity column so as to fixate said biotinlyted recombinant protein in said affinity column.

17(original). The method as claimed in claim 15, wherein the modification of said recombinant protein comprising the steps of:

- obtaining a solution containing the recombinant protein;
- capturing said recombinant protein by using the affinity column so as to fixate said recombinant protein in said affinity column; and
- adding a biotinylation reagent to said affinity column to cause biotinylation reaction with said recombinant protein fixated in said affinity column.

18(original). The method as claimed in claim 16, wherein said recombinant protein is exchanged from the affinity column by a decoupling reagent, said decoupling reagent is chosen according to the properties of the specific tag and the affinity column.

19(currently amended). The method as claimed in claim 18, when said specific tag is Histidine tag and the affinity column is a metal chelating column, the decoupling reagent is ~~imidazole~~imidazole.

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20(original). The method as claimed in claim 18, when said specific tag is maltose-binding tag and the affinity column is an amylose column, the decoupling reagent is maltose.

21(original). The method as claimed in claim 18, when said specific tag is GST tag and the affinity column is a glutathione column, the decoupling reagent is glutathione.

22(currently amended). The method as claimed in claim 1, wherein the immobilization of said recombinant protein is achieved by modifying the recombinant protein with biotin and attaching the biotin-modified recombinant ~~protein~~protein on a substrate coated with streptavidin.